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(54) **Use of molecular markers in tree breeding.**

(57) RFLP technology is applied to tree material from a number of trees and an assessment is made from the RFLP data of the degree of genetic relatedness or diversity of the trees. Thus trees may be selected for crossing or cloning.

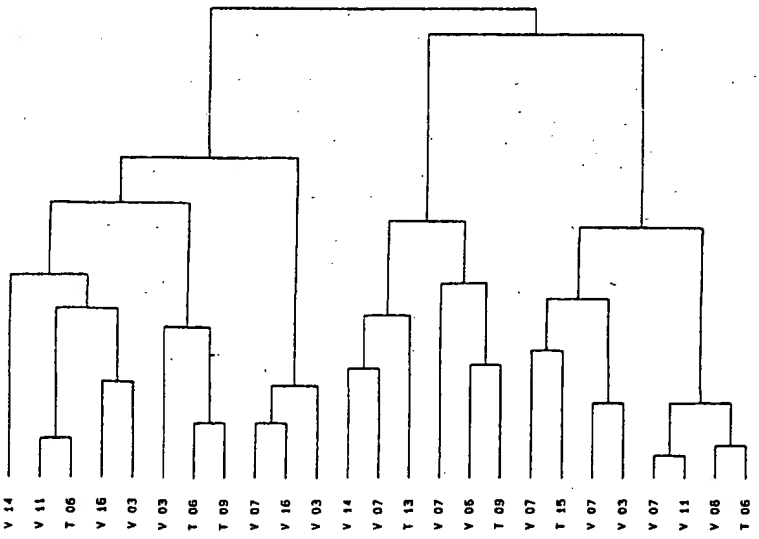

Figure 2

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The potential use of molecular markers for use in plant breeding has been discussed at length (Burr et al 1983; Tanksley 1983; Beckmann and Soller, 1986) and in a number of annual crops such as corn (Helentjaris et al 1986), tomato s (B rnatzky and Tanksley 1986; Hel ntjaris et al 1986), lettuce (Landry et al 1987), and rice (McCouch t al 1988) chromosome maps have been constructed using a combination of RFLPs and isozyme loci. These maps and/or associated molecular probes (Patents WO 84/04758; EP 242062; WO 89/07647) have been used to identify and thereby protect inbred lines or varieties; to breed for quantitative or polygenic traits; for selection of specific traits in segregating seedling populations; and to determine phylogenetic relationships within genera and species.

The improvement of woody tree species presents special problems (Zobel and Talbert, 1984), amongst which are poorly characterised germplasm, a restriction to half-sib analysis of progeny trials from random matings in seed orchards and variable seed quality from year to year.

One technique which has been used in an attempt to overcome some of these problems is isozyme analysis. This has been used for determining genetic relatedness of trees and as a means for assessing degree of selfing and hence potential quality of seedlots (Adams 1983). However, this method suffers from the drawback that although differences between isozyme patterns indicates a true difference, similarity in pattern does not necessarily imply overall genetic similarity as unique isozyme markers may not always be present.

One area where RFLP technology has been applied to a forestry problem was the use of restriction fragment analysis of chloroplast DNA to determine the degree of introgression in natural seedlots of geographically overlapping populations of two spruce species (Szmidt 1988). However, this approach is limited by its assessment of only a small part of the genome which may not reflect diversity at or below the species level.

The use of total genomic DNA for the genetic description of natural populations of two tree species and their interspecific hybrids using RFLP technology has also been studied (Keim et al 1989).

An object of the present invention is to provide a method by which trees can be selected based not only on phenotypic characteristics but also on genetic diversity. This allows the tree breeder to construct more efficient crossing programmes and to ensure maximum genetic diversity in their breeding populations. This gives an economic gain based on a greater proportion of vigorous high yielding trees produced from the collected seed.

In clonal forestry plantings this invention can be used to select clones based on their genetic diversity rather than just performance. Lack of diversity in clonal plantings may lead to catastrophic losses from adverse conditions affecting all similar clones to the same extent at the same time, whereas in plantings selected on genetic diversity these risks are reduced as truly different clones may react in different ways to adverse conditions. For example pest or disease resistance may be present in some clones and not in others. Defined levels of diversity, in terms of clone numbers per unit area, is mandatory to clonal planting in some countries of the world. This invention would allow diversity to be measured on real differences rather than on numbers which may have no significance if all selected clones have similar genetic backgrounds.

The present invention also has as an objective the provision of a method for analysing progeny produced from a crossing programme. In a random mating crossing programme as for example in a seed orchard situation, seed collected from individual trees will have known maternal parents but a number of unknown paternal parents. General combining ability can be estimated from these half sib family progeny trials but no measure of specific combining ability can be obtained. This invention allows for identification of most likely paternal parents of individual trees within half sib families thereby facilitating a more complete full sib family analysis which can give estimates of specific combining abilities of trees within a seed orchard. In a more practical situation identification of both parents of superior individuals within progeny trials can be identified thereby providing useful information for parental selection for further crosses.

Poor seed quality in terms of a high proportion of seed derived from a few trees (selfing) or from too many trees with poor general combining ability can greatly reduce the economic viability of a forestry operation. In addition seed collected from seed orchards may be of variable quality during the productive life of a seed orchard. In young seed orchards only a few precocious individuals may be represented as pollen parents and even in mature seed orchards s ed quality may vary from year to year depending upon the flowering conditions. A further object of this invention is therefore to provide a means of assessing seed quality in terms of probable number and identity of pollen parents represented in a seedlot collected from a seed orchard.

The present invention is of advantage in the breeding of commercial tree types, for example forest trees (Eucalyptus, Pinus, Picea and Populus for instance), perennial plantation crop trees (Elaeis, Cacao, Hevea and Musa for instance) and fruit or nut trees (Malus and Juglans for instance).

According to a first aspect thereof, the present invention provides a method of tree breeding wherein Restriction Fragment Length Polymorphism (RFLP) technology is applied to samples of tree material from a plurality of trees; the information derived from the RFLP technology is presented in a genetic relatedness hierarchy, a level, e.g. the lowest, in said hierarchy comprising groups, each of which group relates two of the trees as being more genetically related to each other than either of the two trees is genetically related to any other tree in any other of said groups; two of said trees of appropriate relative genetic diversity are selected; and a further tree or trees is/are derived from the two selected trees.

A further tree(s) may be derived by crossing the selected pair of trees. Alternatively, further trees may be derived by cloning each of the two selected trees.

In a practical application of the first aspect of the invention with a view to deriving progeny by crossing, the RFLP technology is carried out so as to select a multiplicity of pairs of parent trees from which to derive the progeny.

A second aspect of the present invention provides a method for use in tree breeding, wherein RFLP technology is applied to material from a progeny tree, to material from the mother tree of said progeny tree, and to material from a plurality of trees which are possible paternal trees in respect of said progeny tree, and data therefrom is subjected to analysis thereby to determine the tree of said possible paternal trees which is the most likely paternal tree in respect of said progeny tree. This method can be used in respect of a multiplicity of mother trees in a stand of trees in order to derive knowledge of the specific combining ability.

The tree material to which the RFLP technology is applied is suitably leaf or shoot material, preferably young leaf or shoot material. Preferably, the DNA extracted for the application thereto of the RFLP technology is total genomic DNA.

A third aspect of the present invention provides a method for use in tree breeding, wherein RFLP technology is applied to seed of a body of seed and data therefrom is used to provide a criterion of assessment of said body of seed. The criterion of assessment can be, for example, the degree of selfing of individual trees or the proportion of potential pollen parents represented in that body of seed. The criterion of assessment for said body of seed can be compared with the same criterion in respect of a further body of seed.

Description of Figures

Figure 1 shows a diagrammatic representation of an autoradiogram of DNA from 25 sample trees digested with restriction endonuclease EcoR I and probed with the insert from clone GLPP063. In total 30 discrete fragments are revealed by this probe across these particular trees, but 32 discrete fragments across all trees tested.

[Table 1 shows the banding pattern data from the autoradiogram of Figure 1, reading down the autoradiogram from larger to smaller sized fragments. The data set for each sample consists of a series of 32 binary digits.]

Figure 2 shows the dendrogram produced by clustering analysis of the data shown in Table 1. Small sub-groups can be seen to be clustering together as a result of a higher degree of similarity between one another than with the other members of the sample population as a whole.

Figure 3 shows the dendrogram produced by clustering analysis of the data produced by several (six) probes. The binary strings are concatenated and read as one large data set (118 digits). Clustering of sub-groups is more defined than when using a single probe.

Figure 4 shows the dendrogram produced by clustering analysis of the fingerprint data of six probes with fusion of the last six clusters to produce 6 genetic groups.

Detailed Description of the preferred method

Genomic DNA Preparation
Genomic Digests and Gel Electophoresis
Southern Blotting
Probe Libraries
Probe Preparation
Probe Labelling
Hybridisation and Autoradiography

Clone Selection
Band Scoring and Analysis
Div rsity Analysis

Genomic DNA Preparation

DNA preparation is based on a modification of the method of Dellaporta et al (1983). 10g samples of each of the candidate Eucalyptus globulus trees, preferably young leaf tissue, were ground with a pestle and mortar with liquid nitrogen for 3 minutes. 5g Polyvinyl Polypyrollidone were added, followed by 95ml cold Extraction Buffer (50 mM EGTA, 60 mM Magnesium Acetate, 500 mM NaCl, 100 mM Tris-Cl, 20 mM DTT, 50 µg/ml Pronase E; pH 8.0), and then 5 ml 20% SDS was added, the whole being mixed well after each addition. DNA extraction was achieved by incubation at 65°C for 10 minutes. 35ml 5M Potassium Acetate was added and the lysate stood on ice for 30 minutes. Following centrifugation at 11,000g for 10 minutes, the supernatant was filtered through one layer of Miracloth, and 70ml Propan-2-ol added. The mixture was held at -20°C for at least 2 hours and then centrifuged at 12,000g for 20 minutes. The DNA pellet was drained well, resuspended in 700 µl 50 mM Tris-Cl/10 mM EDTA; pH 8.0, and transferred to a microfuge tube. Insoluble debris was removed by centrifugation for 2 minutes. 500 µl Propan-2-ol and 70 µl 10M Ammonium Acetate was added to the supernatant which was then stood on dry ice for 30 minutes. DNA was pelleted by centrifugation for 10 minutes, drained, and resuspended in 200 µl TE (10 mM Tris-Cl, 1 mM EDTA; pH 8.0).

Genomic Digests and Gel Electophoresis

Restriction digestion of genomic DNA was performed with a variety of restriction endonucleases under conditions and in buffers recommended by the suppliers (Life Technologies).

5 µg of each digested DNA sample was loaded on a 0.8% agarose gel in TBE buffer (90 mM Tris, 90 mM Boric Acid, 2 mM EDTA). 400 ng of a Hind III digest of lambda DNA was loaded on the same gel to act as a size standard. Digests were electrophoresed overnight at approximately 1.3 V/cm.

Southern Blotting

All blots were prepared by capillary transfer by a procedure similar to that of Southern (1975) as described by Sambrook et al (1989a), with the following modifications; (i) the blotting membrane used was Biodyne B (0.45 µm, nylon), (ii) this membrane does not require the gel to be neutralised after the alkaline denaturation step, and, (iii) following transfer, the membrane was baked at 80°C for 30 minutes, and then rinsed in 2xSSC solution (1xSSC is 150 mM NaCl, 15 mM Sodium Acetate; pH 7.2), wrapped in Saran Wrap, and stored at -20°C till required.

Probe Libraries

All the clones used were derived from two E. globulus genomic libraries. Total genomic DNA from E.globulus was prepared as described above, restricted by Pst I, and cloned into plasmid vector pUC9. The clones were maintained as bacterial glycerol cultures at -80°C, and when required grown up on L-agar containing 100 µg/ml ampicillin.

Probe Preparation

The probe DNA was prepared by Polymerase Chain Reaction (PCR) amplification of the genomic DNA insert in each plasmid clone. This was achieved by suspension of a bacterial colony containing the clone of choice directly in the PCR reaction buffer.

Amplification of the clone inserts was performed with Taq Polymerase in buffer provided by the supplier (Promega) and supplemented with four deoxynucleotide triphosphates (dATP, dGTP, dCTP, dTTP; 200 µmol each), and two oligonucleotide primers (0.25 µg per reaction), in a reaction volume of 50 µl containing 0.25 units of Taq Polymerase. Incubation was performed with the following programme;

94°C x 90sec, 40°C x 10 sec, 70°C x 3 min; 1 cycle
94°C x 10sec, 40°C x 10 sec, 72°C x 3 min; 9 cycles
94°C x 10sec, 40°C x 10 sec, 72°C x 4 min; 10 cycles
94°C x 10sec, 40°C x 10 sec, 72°C x 5 min; 10 cycles

72°C x 5 min; 1 cycle

The following primers were used during PCR;

RFLP1 ATTACGCCAAGCTTGGCTGCA

RFLP2 TTCCCGGGGATCCGTCGA

M13 Sequencing and Reverse Sequencing primers could also be used for PCR with all clones.

Following PCR and gel analysis the clones were purified by phenol/chloroform extraction, followed by ethanol precipitation. It was possible to omit the phenol/chloroform extraction step without deleterious effects.

Following DNA estimation by fluorometry (Hoeffer fluorometer) 1ng of lambda DNA was mixed with 50 ng of each insert to highlight the size standards on the genomic blots.

Probe Labelling

DNA probes were radiolabelled by the random hexamer method of Feinberg and Vogelstein (1983, 1984) using a Pharmacia Oligonucleotide Labelling Kit. 50 μCi of ^{32}P-dATP were used to label 50ng of probe DNA. After incubation for 2 hr the reaction was stopped and the probe denatured by adding 200 μl water and boiling for 3 minutes. The probe was then immediately placed on ice and added to the hybridisation solution within 10 minutes.

Hybridisation and Autoradiography

The genomic blot to be hybridised was pre-washed in 2xSSC, then in 1% Triton X-100, and lastly Prehybridisation Solution (detailed below). All solutions were pre-warmed to 55°C. The membrane was then placed in hybridisation bottles with 50-100ml Prehybridisation Solution (5 x SSC, 50 mM Tris-Cl, 5 mM EDTA, 0.2% BSA, 0.2% Ficoll 400,000, 0.2% Polyvinyl Pyrollidone, 0.1% SDS, 0.1% Sodium Pyrophosphate, 0.05% "Blotto"; pH 7.5) and incubated at 55°C in a rotary oven (Hybaid) for at least 5 hours (usually overnight).

The Prehybridisation Solution was discarded and 20ml of fresh solution added (pre-warmed to 55°C). The denatured probe was then added and the bottle replaced in the hybridisation oven and incubated at 55°C for a time determined by the size of the probes and calculated as being within the range 1-3 x $cot_{1/2}$.

Following hybridisation the membrane was washed at 55°C in a shaking waterbath sequentially in the following 3 solutions; (i) 1l 2xSSC/0.2%SDS for 1 hr, (ii) 1l 0.5xSSC/0.2% SDS for 1 hr, (iii) 1l 0.2xSSC/0.2%SDS for 30 minutes. The membrane was then wrapped in Saran Wrap and autoradiography performed as described by Sambrook et al (1989b).

Following autoradiography, the membrane was stripped for re-probing by incubating with shaking in the following solutions; (i) 0.4M NaOH at 45°C for 30-45 minutes, (ii) 0.1xSSC/0.1%SDS at room temperature for 15 minutes, (iii) 0.2M Tris-HCl pH7.4 at room temperature for 15 minutes. The membrane was then covered with Saran Wrap and stored at -20°C for re-use.

Clone Selection

Clones from the E.globulus libraries were hybridised against Southern blots of restricted genomic DNA from a small number of Eucalyptus species, interspecific hybrids, and E.globulus provenances. Those clones which showed the highest degree of discrimination between these samples, either individually or in concert, were selected for use in the subsequent analysis of a wider range of E.globulus provenances.

Band Scoring and Analysis

On development of the autoradiograms the hybridising fragments (bands) present from each genomic DNA were scored as follows. The position of all bands on each autoradiogram (or each set of autoradiograms when several membranes had been hybridised with the same probe) were located with reference to the lambda DNA size standards. Each genomic DNA was then scored for the presence or absence of each of these bands, the result being recorded as "1" (present) or "0" (absent). The band pattern for each genomic DNA (and thereby each tree) was described by a series of binary digits e.g. the pattern 10010111001 might describe a genomic DNA which exhibited 6 fragments hybridising to a probe which revealed a total of 11 discrete fragment sizes across the whole range of DNAs (trees) scre ned in this way.

Data obtain d from several probes could be combined by appending the binary sequence from each subsequent probe/autoradiogram to produce a long r string of digits. No attempt was made to add weighting to any of the data to reflect different intensities of the bands, or to allow for th fact that different probes revealed the presence of diff rent numbers of bands. When groups of trees were compared, the data from all possible fragments were scored including common bands (present in all cases) and bands absent on individual autoradiograms but found within the group (of trees) on other autoradiograms. This ensured that the fragment pattern of an individual tree was the same irrespective of which other trees were included on the autoradiogram. This ensured that the degree of similarity between any two trees took the same value irrespective of which other trees were also in the analysis.

A "genetic fingerprint" can then be constructed for each tree using the fragment patterns from a number of probes.

Diversity Analysis

A similarity matrix was computed using similarity coefficients such as Jaccard's Coefficient (Sokal and Sneath, 1963) which uses the number of matching bands between any two samples divided by the total number of matching and unique bands present in the two samples. These coefficients can then be used in a clustering algorithm, for example Unweighted Pair Group Arithmetic Average Clustering Method (UPGMA) or Ward's Method (Wishart, 1987) to group the most similar samples together in dendrograms or other graphical representations.

An example is now given of use of these fingerprints to measure genetic diversity in a commercial tree breeding population.

EXAMPLE

DNA was extracted from 92 randomly selected trees from an E.globulus provenance trial, of known seed origin, in a commercial breeding programme. The origin of this material was from seed collected in 32 locations in the Australian states of Victoria and Tasmania and its nearby islands. Genetic fingerprints were produced with 14 selected probes for each tree as described above. Similarity matrices, using Jaccard's Coefficient of Similarity, were computed and dendrograms produced using Ward's Method for individual probes (an example of the autoradiogram of 25 trees probed with a single probe, GLPP063 and the fingerprint as presence or absence of bands are shown in Figure 1 and Table 1 respectively). The fingerprints from the most discriminatory probes were then combined sequentially and further similarity matrices computed. Once stable groupings were produced (Figure 3), which, in this case was with 6 probes, major groups were assembled (Figure 4). The six probes were GLPP011; GLPP029; GLPP063; GLPP093; P002 and P022. A sample of each of these six probes, harboured in E. Coli, was deposited under the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the Purpose of Patent Procedure, at the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland on 17th September 1991 under the respective accession numbers NCIMB 40444; NCIMB 40443; NCIMB 40442; NCIMB 40441; NCIMB 40446 AND NCIMB 40445.

In this example, trees in Groups 2 and 3, as a whole, were more dissimilar, that is to say genetically diverse, compared with trees in Groups 1, 6, 4 and 5 and within this latter grouping, trees in Groups 1 and 6 were genetically more diverse than trees in Groups 4 and 5.

It can be seen from Table 2 that Groups 2 and 3 consist solely of Victorian provenance material and that none of these provenances was split between the 2 groups so that Group 2 comprised of 20 trees from 4 different provenances and Group 3 of 24 trees from 8 provenances. The classification for Groups 1, 6, 4 and 5 was not so clear but overall these 4 groups consisted of the Tasmanian provenances and Groups 1 and 6 formed one set with 25 trees from 12 Tasmanian provenances and Groups 4 and 5 another set with 16 trees from 4 other Tasmanian provenances. Only one Tasmanian provenance (T 08) had trees in these two sets. There were, however, 4 Victorian provenances in these two sets, one of which (V 03), was represented by 4 trees which would strongly suggest an unusual origin for this particular provenance.

The discrimination between the Tasmanian and Victorian provenances was not surprising but the degree of discrimination of provenances within these two geographical regions was surprising. What is more, this type of information on g netic diversity can be used by tree breeders to organis their germplasm and help in the decision of what crosses to make or clones to select. Crosses between trees in Groups 1, 6, 4 and 5 and trees in Groups 2 and 3 would create the widest genetic base and crosses within

the same groups would significantly reduce genetic diversity. Likewise, clones selected from across all the different Groups would give a wider genetic diversity of planting material than clones selected from within one Group.

This can also be used to measure genetic diversity in natural populations as a means to either deciding which areas contain the most diverse populations or which particular genotypes should be conserved.

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Table 1

Fingerprints as presence (1) or absence (0) of bands from one probe GLPP063 for 25 trees from Tasmanian and Victorian provenances.

BAND	PATTERN
V14	0000010001001000000000000000000
V14	0000000000001010101000101000000
V07	1000000000001000100000100000000
V07	0000000001001010100001100000000
V07	0001001011101000100001100000100
V07	0000000100001101110001100011010
V07	0000000001101000100001100000000
V07	0000001100001010100000101100000
V11	0000000000001010100001100000000
V11	0000010001011000100001110000100
V06	0100001100011000111100100000000
V06	0000000000001010100000100000000
V16	0000011011101000100001100000000
V16	0000010101001000100111100000000
V03	0000101011101100100011100000000
V03	0000000001101000100000000000000
V03	0000010001001100100100100000000
V03	0000001101001010100000100000111
T06	0000001001001000100101100000100
T06	0000000001001010100000100000000
T06	0000010001001000100001100000100
T09	0000001001001010110101101000100
T09	0000001100101000110000100000000

T13 0000000100110110100000101000000

T15 0000000001001001100000100000000

TABLE 2

Distribution of trees in 6 Groups for E.globulus Tasmanian provenances (T 01 - T 15) and Victorian provenances (V 01 - V 17).

GROUP 1	GROUP 6	GROUP 4	GROUP 5	GROUP 2	GROUP 3
T 01	T 01	T 05	T 05	V 07	V 01
T 03	T 02	T 06	T 05	V 07	V 02
T 03	T 04	T 06	T 05	V 07	V 04
T 04	T 08	T 06	T 05	V 07	V 04
T 04	T 11	T 06	T 05	V 07	V 04
T 09	T 12	T 06	T 05	V 07	V 04
T 09	T 12	T 07	T 08	V 07	V 04
T 09	T 12	T 07		V 11	V 04
T 10	T 12	T 07		V 11	V 04
T 11	T 13	V 03		V 11	V 06
T 14	T 15	V 03		V 11	V 06
T 15	T 15	V 03		V 11	V 06
T 15		V 03		V 11	V 06
V 12		V 05		V 11	V 08
V 14				V 11	V 08
				V 11	V 08
				V 11	V 09
				V 14	V 09
				V 14	V 16
				V 15	V 16
					V 16

Table 2 Continued

	V 16
	V 17
	V 17

Claims

1. A method of tree breeding wherein Restriction Fragment Length Polymorphism (RFLP) technology is applied to samples of tree material from a plurality of trees; the information derived from said RFLP technology is presented in a genetic relatedness hierarchy, a level in said hierarchy comprising groups, each of which groups relates two of said trees as being more genetically related to each other than either of the two trees is genetically related to any other tree in any other of said groups; two of said trees of appropriate relative genetic diversity are selected; and a further tree or trees is/are derived from the two selected trees.

2. A method according to Claim 1, wherein a further tree is derived by crossing the two selected trees.

3. A method according to Claim 1, wherein further trees are derived by cloning each of the two trees.

4. A method according to Claim 1, 2 or 3, wherein said trees are of a commercial species.

5. A method according to Claim 4, wherein said species is a Eucalyptus species.

6. A method according to any preceding claim, wherein said samples are leaf samples.

7. A method according to any one of Claims 1 to 5, wherein said samples are shoot samples.

8. A method for use in tree breeding, wherein RFLP technology is applied to material from a progeny tree, to material from the mother tree of said progeny tree, and to material from a plurality of trees which are possible paternal trees in respect of said progeny tree, and data therefrom is subjected to analysis thereby to determine the tree of said possible paternal trees which is the most likely paternal tree in respect of said progeny tree.

9. A method according to Claim 8, as applied in respect of a multiplicity of mother trees in a stand of trees.

10. A method for use in tree breeding, wherein RFLP technology is applied to seed of a body of seed and data therefrom is used to provide a criterion of assessment of said body of seed.

11. A method according to Claim 10, wherein the criterion of assessment is the degree of selfing of individual trees.

12. A method according to Claim 10, wherein the criterion of assessment is the proportion of pollen parents represented in said body of seed.

13. A method according to Claim 10, wherein the criterion of assessment of said body of seed is compared to the same criterion established in respect of a second body of seed.

14. A method according to any one of the preceding claims, wherein the probes used in the RFLP technology comprise one or more of GLPP011; GLPP029; GLPP063; GLPP093; P002 AND P022.

15. An RFLP probe comprising GLPP011.

16. An RFLP probe comprising GLPP029.

17. An RFLP probe comprising GLPP063.

18. An RFLP probe comprising GLPP093.

19. An RFLP probe comprising P002.

20. An RFLP probe comprising P022.

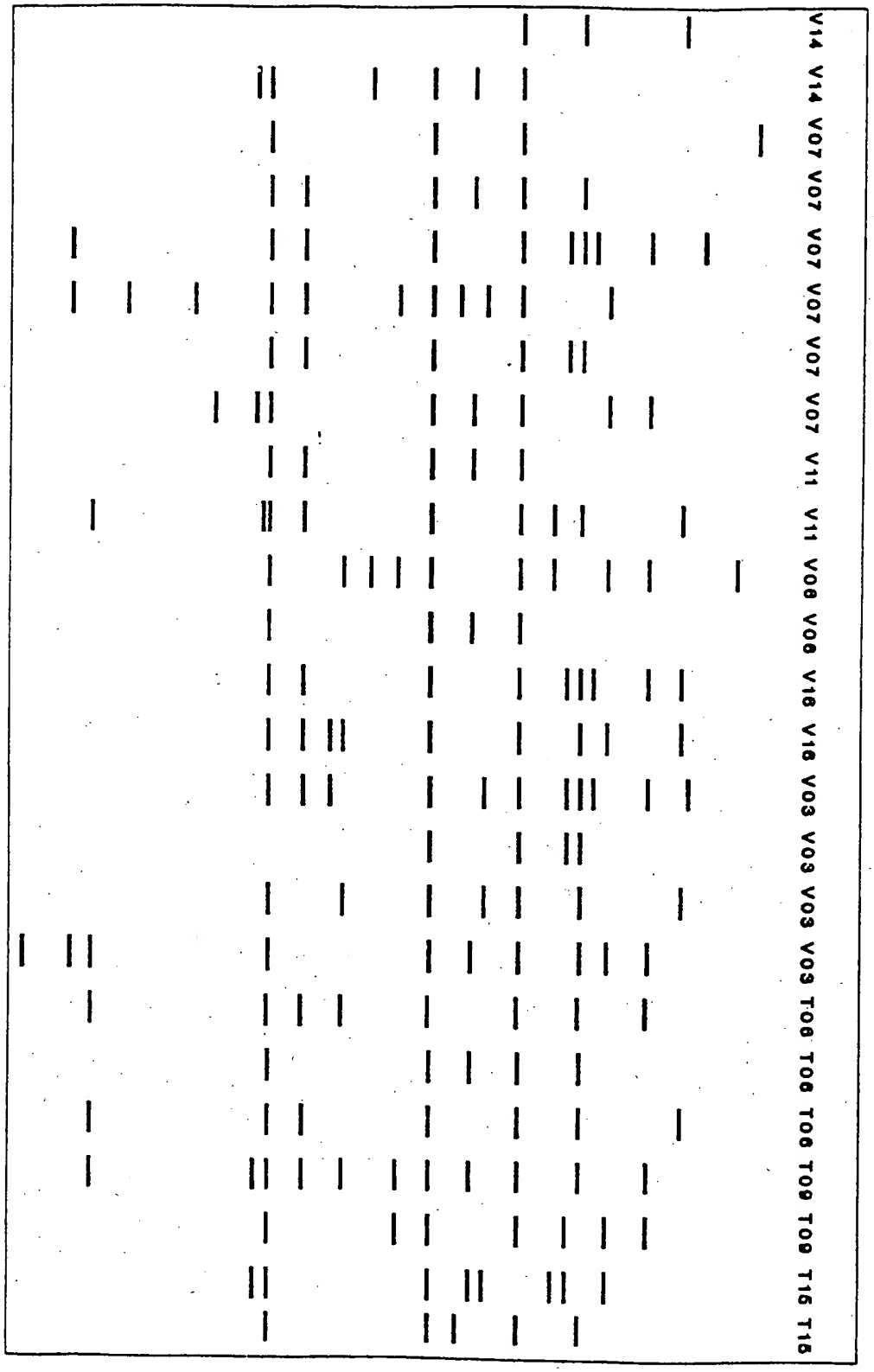

V14
V14
V07
V07
V07
V07
V07
V11
V11
V08
V08
V16
V16
V03
V03
V03
V03
T06
T06
T06
T09
T09
T15
T15

Figure 1

Figure 2

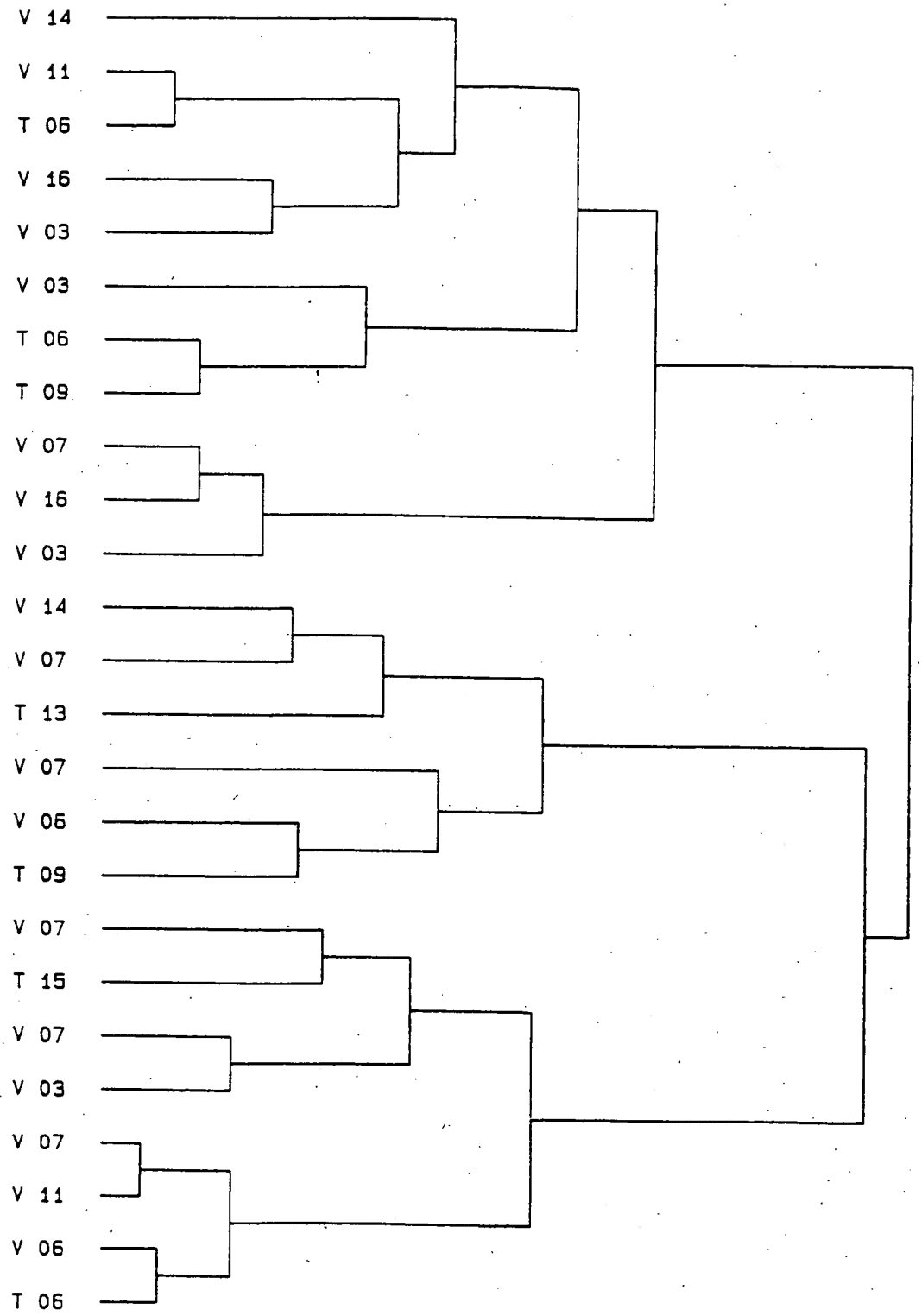

V 14
V 11
T 06
V 16
V 03
V 03
T 06
T 09
V 07
V 16
V 03
V 14
V 07
T 13
V 07
V 06
T 09
V 07
T 15
V 07
V 03
V 07
V 11
V 06
T 06

Figure 3.

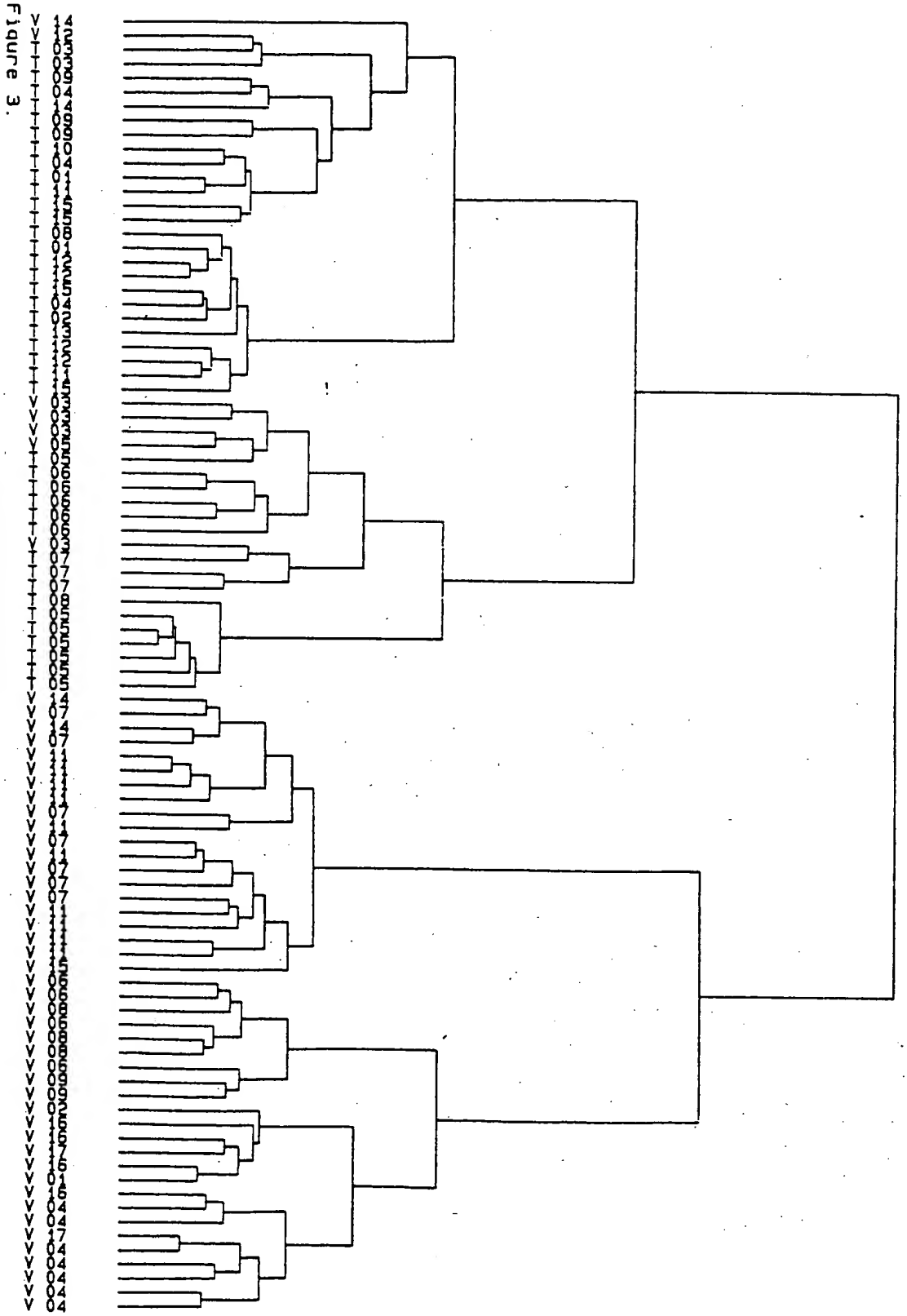
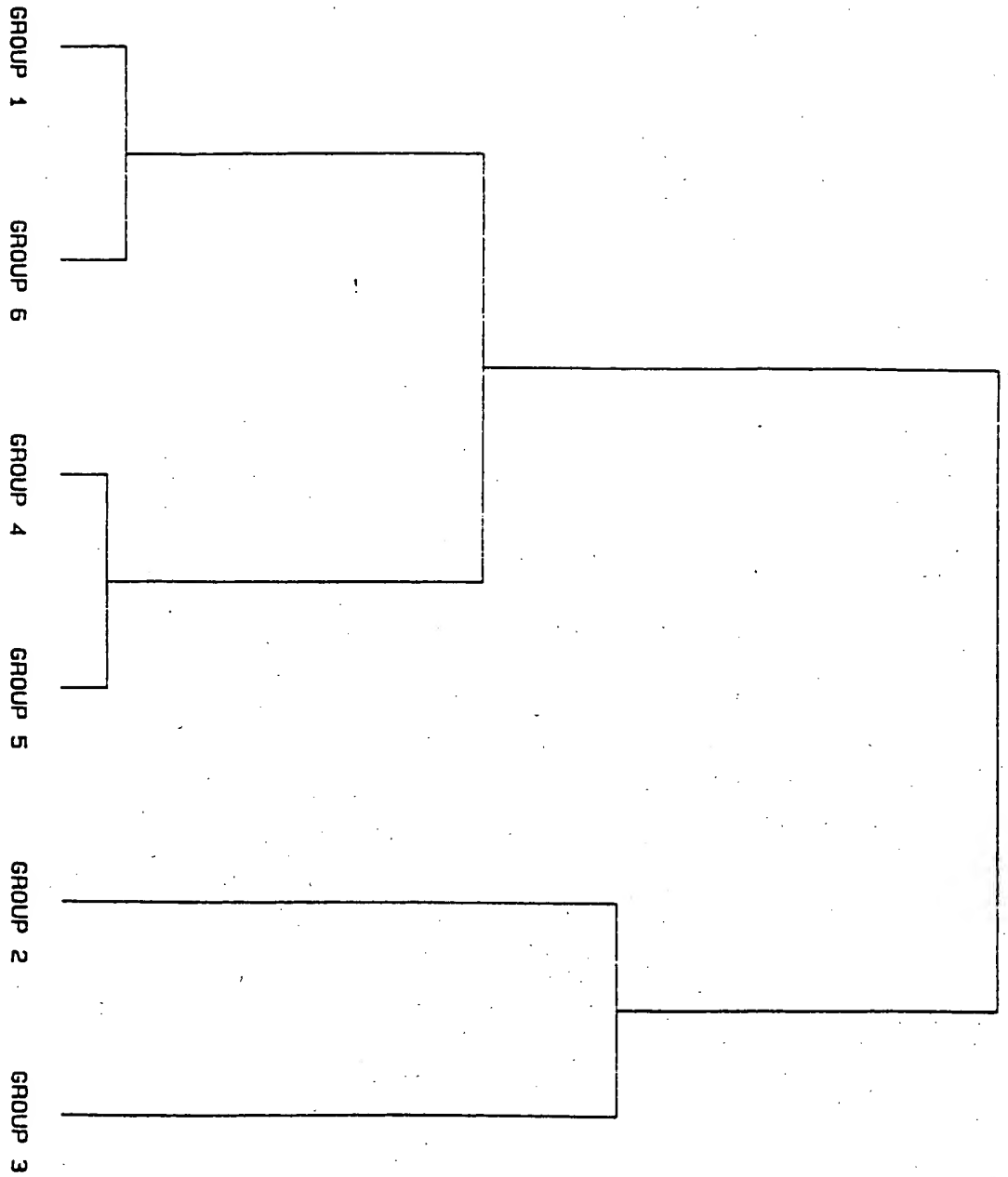


Figure 4



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PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number

EP 91 11 6407

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X	GENETICS, vol. 123, November 1989, pages 557-565, The Genetics Society of America, Austin, Texas, US; P. KEIM et al.: "Genetic analysis of an interspecific hybrid swarm of populus: occurrence of unidirectional introgression" * Whole document, especially abstract * ---	1,8	C 12 Q 1/68 C 07 H 21/04
D,X	THEOR. APPL. GENET., vol. 76, 1988, pages 841-845, New York, US; A.E. SZMIDT et al.: "Classifying seedlots of Picea sitchensis and P. glauca in zones of introgression using restriction analysis of chloroplast DNA" * Whole document, especially abstract * --- -/-	1-3,8-10	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 12 Q

INCOMPLETE SEARCH

The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims

Claims searched completely: 1-13

Claims searched incompletely:

Claims not searched: 14-20

Reason for the limitation of the search:

Insufficient support of the claims by the description.

Place of search	Date of completion of the search	Examiner
THE HAGUE	19-12-1991	MOLINA GALAN E.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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& : member of the same patent family, corresponding document

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PARTIAL EUROPEAN SEARCH REPORT

Application Number EP 91 11 6407

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
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X	PLANT BIOLOGY, HORTICULTURAL BIOTECHNOLOGY, vol. 11, 1990, pages 335-346, New York, US; M. WALTON: "Application of RFLP technology to applied plant breeding", & HORTICULTURAL BIOTECHNOLOGY SYMPOSIUM, Davis, CA, 21st - 23rd August 1989 (Cat. O) * Abstract * ---	1-3,8-10	TECHNICAL FIELDS SEARCHED (Int. Cl.5)
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A	WO-A-8 404 758 (PLANT RESOURCES INST.) ---		
A	PLANT BIOLOGY, HORTICULTURAL BIOTECHNOLOGY, vol. 11, 1990, pages 247-259, New York, US; J.W. DeVERNA et al.: "RFLP technology" ---		
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